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MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA

RANGPUR TOBACCO WILT

BY

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AGRICULTURAL RESEARCH INSTITUTE, PUSA

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IN February 1911 diseased tobacco plants of Sumatra and local varieties were received at Pusa from Rangpur. The plants had wilted in the field, the lower leaves being blackened and the withering progressing upwards. The Imperial Mycologist having examined the specimens and reported them free from fungi but full of bacteria, they were transferred to the Bacteriological Section.

Plate I shewing a longitudinal section through the stem gives a good idea of the condition of the plants when received. It will be seen that the browning of the tissues, which is the result of bacterial action, spreads from the "collar" probably the point of infection, up into the stem and along the leaf traces, discolouring the pith. The roots in this specimen were poorly developed, probably in consequence of bad cultivation and drainage, and exhibited internal discoloration.

Erwin F. Smith has described a similar disease of tobacco in America (The Granville Tobacco Wilt, U. S. Department of Agriculture, Bulletin No. 141, 1908), and shewn the causative organism to be B. solanacearum; this bacterium is also known to be pathogenic for other solanaceous plants such as tomato, brinjal (Solanum Melongena), potato and *datura*, and has been found by Coleman producing disease in potatoes in Southern India.

This disease occurs annually in the Rangpur district and has been known for many years under the name of "Rasa." Its distribution in the field is irregular and patchy, and I have not been able to trace any connection between soil conditions and incidence,

beyond the general lack of good root development in diseased plants. The cultivators express but little anxiety over the occurrence of the disease, regarding it as an inevitable dispensation of Providence, and their attitude of indifference is explained by the generally low average of cases, which seldom amounts, so far as I was able to ascertain, to more than 5 or 6 per cent., although I saw fields in which as many as 20—25 per cent. of the plants were affected. It is of course possible that in any year conditions favourable to the disease might produce a heavy mortality, and a succession of such seasons, by accumulation of infective material and increase in virulence, might result in the conditions now obtaining in parts of North Carolina and Florida, where tobacco has been so severely affected that it is found impossible to grow this crop owing to the persistence of infection in the soil, which continues for many years.

The stems of the diseased plants received from Rangpur when cut across the darkened portions exuded a greyish white slime, which under the microscope was found to be full of bacteria, varying in morphological characters.

In order to determine whether these were merely saprophytes or contained amongst their number a pathogenic organism causative of the disease, it was necessary to make pure cultures of all the bacteria present and test their possible pathogenicity on healthy plants. This operation was found extremely tedious owing to the large number of species present and the fact that the latter varied in different specimens. Plate cultures demonstrated the presence amongst others of the following saprophytic forms. B. megatherium, B. subtilis, B. prodigiosus, B. mesentericus, B. fluorescens, besides many others unidentified; the presence of B. prodigiosus was of interest as I had failed up to that time to find this species in any Bengal soils; and it only occurred in one of the diseased specimens examined.

Various methods of obtaining cultures containing fewer species were adopted; the original method of flaming the infected stem and cutting out externally uncontaminated blocks with a red hot

knife * was superseded by the following which reduced the number of different organisms to some three or four on each plate. That part of the stem of the diseased plant was selected just outside the limit of the spread of the externally visible discolouration. A section of the previously flamed stem was cut out with a red hot knife ; a red hot needle was driven into the junction of the discoloured and of the unaffected pith. The block was then flamed once more and rapidly dipped into melted paraffin wax at 80° C., so as to coat the whole surface together with part of the needle, by which latter the block was held with forceps ; the wax coat was allowed to harden and the flaming and dipping repeated twice. The waxed end from which the needle protruded was then flamed rapidly, the needle withdrawn and the waxed block dropped into bouillon, which entered into the puncture and penetrated through the portion sterilized by the entry of the hot needle, the result being a culture containing a greatly reduced number of organisms. From this culture plates were made and five organisms brought into pure culture.

Previous to the adoption of this method numerous cultures had been made from the bacterial slime from diseased plants, and also from blocks cut from diseased stems with a hot knife. These cultures were found to be largely B. mesentericus, various spore forming rods, and but few cocci, but the recurrence of one form in a large number of plates led to its closer investigation. It was first noticed that this organism, which, in one or two preparations direct from the diseased stem of the plant, largely preponderated over all others, was distinguished by a tendency to bipolar staining with methylene blue, and this character was subsequently found useful for recognizing its presence in mixed cultures. Examination of sections of diseased tissue afforded but little help in determining the pathogenic organism, as although many of the vessels were full of bacteria, the latter presented such a variety of morpho-

* This method was originated by Dr. Butler, Imperial Mycologist, and successfully utilized by him in isolating the fungus causing red rot in sugarcane in India.

logical characters, both in dimensions, outline, motility and the reverse, that no definite idea could be obtained from such inspection.

Inoculations into healthy plants with bacterial slime from diseased specimens were by no means generally successful, in fact the first successful inoculations were obtained with pure cultures either on potato or agar, the former yielding the greater percentage of positive results. No inoculations from bouillon were successful in early stages of the inquiry, probably owing to the preponderance of saprophytes in these impure cultures.

It was found that a ready means of distinguishing bouillon cultures containing a sufficient preponderance of the pathogenic organism to serve as efficient sources for inoculation, from those in which the pathogen had been overcome by competition with numerous saprophytes, existed in the alkaline reaction characteristic of the former : a rough test with litmus also served to distinguish fungal disease of tomatoes in the field from that caused by B. solanacearum. Plate II shows the effect of inoculation with a pure culture of B. solanacearum on green tomato fruit, which ripened in two days' time, the control fruit on the same stem requiring another fourteen days to attain the same condition. Tests with litmus showed the juice of the inoculated fruit to be strongly alkaline on the side next the point of inoculation, and acid on the opposite side.

An explanation of the presence of such a large variety of saprophytes was subsequently discovered in the fact that a plant the vitality of which had been lowered by infection with the pathogenic bacterium or by other causes, was liable to invasion by saprophytes, either through the original point of entry of the former, or possibly through the leaf scars on the stem resulting from the dropping off of the lower leaves. These leaf scars may possibly afford points of ingress for the parasitic bacterium in the first instance, as in many cases the leaf is broken off or drops before completion of the absciss layer, leaving a raw unprotected surface such as may be seen near the base of the stem of the healthy plant

in Plate III, and are the result of normal action, accelerated in many instances by lack of moisture in the soil, such as would occur in badly cultivated soils, where the water-supply during the cold dry weather was insufficient to balance the loss by transpiration during the heat of the day. Leather has shewn (Water Requirements of Crops in India : Memoirs of the Dept. of Agri. in India, Chemical Series, Vol. I, No. 8, 1910) that a serious loss of water from the soil takes place during the growth of "Rabi" (cold weather) crops, of which tobacco is one. The seed is sown in August or September in seed beds, and the seedling is transplanted into the field in September, October or November, the crop being harvested in the following spring. From November until early February, the time of harvesting, the climate of the tobacco growing districts in North East India is practically rainless, so that the plant depends for the large amount of water transpired through its great extent of leaf surface upon supplies already stored in the soil, or upon dew, as irrigation, although practised in some districts, is by no means universal. Such soil water-supply depends upon efficient tillage, and it therefore follows that this aspect of cultivation not only affects the general yield of the crop, but also its power of resisting the attacks of parasitic bacteria, and with them of saprophytes. It is interesting to note that the local (Rangpur) name for this disease is "Rasa" or "Moisture" disease, the ryots attributing it to excess of moisture in the soil. Mr. C. C. Ghosh, Assistant to the Imperial Entomologist, who investigated the question from the entomological point of view, and to whom I am indebted for much useful and accurate information as to the local conditions, which I was able to confirm when I subsequently visited the district, mentions this fact in his report, but points out that the local methods of cultivation are actually conducive to the conservation of soil moisture. It is a notable case of empiricism in agricultural practice, as I found on enquiry that the ryots of Rangpur are universally of opinion that the disease is due to excess of moisture and that their methods of cultivation, involving repeated shallow ploughing and hand hoeing, tend to dry the soil, whereas the actual

result is the formation of a surface mulch with consequent conservation of soil moisture. At the same time the drying out of the surface soil and its exposure to the high sun temperature would go far towards freeing it from B. solanacearum, so that this method of cultivation, arrived at empirically, is a perfectly sound and recommendable one for the purpose for which it is intended by the ryot. I have been unable to produce infection by watering the soil with cultures of the pathogenic bacillus except when previous injury to the roots had taken place, as might occur, and probably does in a large number of cases in transplanting. It also appears probable that many of the specimens submitted had been attacked by boring insects or nematodes, which by wounding the roots or the underground stem afforded points of entry for the bacterium. (See Plate I.) A careful enquiry into this aspect of the case was undertaken by Mr. C. C. Ghosh, but from his report it would appear that no such source of injury was to be found in the affected areas examined by him, although nematodes may have been present in the soil at the time of transplanting, or in the seed bed; none were found in the diseased plants in 1910 or 1911, although it is quite possible that they may have escaped observation, as in the present season (1912-13) nematodes have been found by the writer in every specimen of diseased plant, of both Sumatrana and local varieties, collected in the Rangpur district (in January 1913) and an obvious connection appeared to exist between their point of entry, generally at or about ground level, and the course of infection in the plant. So far I have not been able to verify this connection experimentally, as although the nematodes found were still alive they were apparently in too lethargic a condition at this time of year (January) to interest themselves in healthy plants presented to their notice. It cannot be doubted, however, that their burrowing habits would lead to the introduction of bacteria present in the soil or on the surface of the stem or roots, into the interior of the latter, and therefore in dealing with this disease such measures should be adopted as would help to eradicate them from tobacco land.

There can be no doubt moreover that given the presence of an infective bacterium in the soil, the ordinary operation of transplanting will invariably afford opportunities of invasion through broken roots, the vitality and resistance of the plant being at the same time lowered by the transference from the seed bed; it is also to be remembered that the common practice of breaking off the lower leaves at the time of transplanting provides numerous points of entry for soil organisms, as was noted previously in the case of the natural dropping of these leaves.

The infecting organism exhibited the following cultural and morphological characters.

Bacterium.—0·6μ—1·0μ to 1·5μ. Motility doubtful, no flagella observed.

Agar slant.—Growth white moist smooth becoming sepia brown; agar stained.

Gelatine.—Colonies round white thin: opalescent: brown under magnification, by transmitted light. No liquefaction.

Bouillon. Turbidity on shaking: alkaline reaction.

Potato.—Growth watery and colourless at first, then opaque white, becoming brown and finally bitumen black.

Glucose bouillon.—No acid or gas.

These cultural characters are identical with those of B. solanacearum Smith as described in U. S. Department of Agriculture Plant Path., Bulletin XII, 1896. The morphological difference existing in the absence of flagella and motility in the Rangpur organism was confirmed by repeated observations extending over more than two years.

In making cultures direct from the slime from diseased tobacco plants, the presence of a motile bacillus was almost invariably noticed. Slides made directly from slime shewed this organism together with non-flagellated B. solanacearum, and cultures on agar and potato depended for their character upon the relative number of the two organisms originally present in the slime; in early stages of the disease B. solanacearum predominated and the cultures

rapidly developed the brown and finally black pigmentation characteristic of this organism ; in later stages of disease the cultures on potato became yellow ochre with a greenish tinge after 48 hours incubation at 30° C., and in this case never subsequently developed brown pigment.

Inoculations made direct from slime varied in their virulence according to the stage of diseased condition reached in the plant. As mentioned above, in advanced stages saprophytes were present in great numbers and variety, and inoculations made with the slime or from mixed cultures made from it, were generally unsuccessful. In earlier stages the slime was almost invariably successful as an inoculum, and invariably when cultures on agar or potato made from it produced brown pigment. At an intermediate stage between these two extremes the occurrence of the yellow pigment-forming bacillus was invariably noticed, and in fact it was almost invariably present in the slime of tobacco plants suffering from this disease, although pure cultures of it had no pathogenic effect, and as already stated, if allowed to grow in competition with E. solanacearum in artificial media of ordinary composition the latter was generally overcome.

The similar morphological characters of these two organisms apart from flagellation, might give rise to the opinion that slime containing them both contained B. solanacearum alone, and that this latter organism was consequently motile. Plate IV, Figs. 1 and 2, shews the two organisms as present in the slime from a diseased tobacco plant.

Plate V shews the growth of B. solanacearum on agar and on potato, both being characterized in the latter stages by the production of dark brown pigment. It was found that when the cultures reached this stage they were no longer pathogenic and in fact in many cases failed to produce growth when transferred to similar media ; pigment production appears with this organism to indicate loss of vitality or even a moribund condition, possibly due to accumulation of excess of metabolic products.

Variations in morphological character and in virulence were found to occur depending upon the culture medium used: thus the cultures on steamed potato produced obvious symptoms of disease in tobacco plants (browning of outside of stem or of midribs of leaves) within from five to eight days when inoculated by a single needle prick into the stem, whereas agar cultures (+5 Fuller) required from eight to twelve days and those in bouillon seldom shewed results under eighteen days. These periods are very much shorter than those observed in the early stages of the inquiry, when as much as five weeks sometimes elapsed between inoculation and appearance of the first symptoms: this was no doubt due to the increase of virulence in the cultures after passing through several plants; the failure of some inoculations direct with bacterial slime from diseased plants was no doubt due to the preponderance of saprophytes in the mixed culture.

Several plants which remained apparently healthy for as long as three or four weeks after inoculation were cut down and sections made from that part of the stem containing the inoculation point. Examination of these sections in every case shewed the presence of B. solanacearum, which on subsequent transference to agar made normal growth and were in some instances successfully utilized for inoculation, but in the tissues thus sectioned the spread of infection had apparently been limited and confined to a radius of a few millimetres by the natural resistance of the plant, or the low degree of virulence of the culture originally used.

It was found impossible to keep the cultures on solid media alive throughout the year except by repeated transfers and storage in the cool incubator at 20°C. The cultures so kept were found in October 1911 to have lost their virulence to such an extent that when inoculated into a young tobacco plant no symptoms of disease appeared until after six weeks. The virulence was gradually regained by passage through a fresh series of plants, but certain alterations in cultural character occurred and were maintained in this second year 1911-12 of growth of the cultures. Thus the characteristic pigment formation, sepia brown on agar, and bitumen black

on potato, was entirely absent, and this variation at first led to doubt as to the purity of the cultures. A point of interest, however, was found in the fact that cultures made on the same media, agar and potato, from fresh specimens of diseased tobacco plants collected by the writer in the same district (Rangpur) in February 1912, exhibited similar characteristics, no pigment being formed by any of these new cultures. It is remarkable that a similar variation was observed by Dr. Coleman, Director of the Mysore State Agricultural Department, who has been working on what appears to be the same organism, in this case causing disease in potatoes. In the year 1910-11 Dr. Coleman's cultures produced brown and black pigment; in 1911-12 no dark pigmentation was observed. No obvious explanation of this variation appears at first sight; two alternatives only suggest themselves, either an actual seasonal periodic variation has occurred, or the cultures made by Dr. Coleman and myself in 1910-11 were not pure. It is not possible to adopt the explanation that the 1911-12 cultures were not so pure as those of 1910-11, as in this case their pathogenicity would have suffered, which did not appear to be the case. It appears probable, however, that this change in cultural character must be due to obscure causes connected with seasonal variation, but it is of special interest in view of the fact mentioned above that in the second season, 1911-12, no pigmentation was observed in the fresh cultures made from diseased plants of that year's growth: had the disease been investigated in India for the first time in 1911 instead of in 1910, the absence of pigmentation would have led to the inference that it was caused by a specific organism differing from B. solanacearum in this respect: diseased plants received in January 1913 from the same locality yielded cultures producing pigment in what appears to be the normal manner.

The morphological characters varied similarly; on potato the bacterium almost invariably was somewhat larger than on agar or in bouillon; the diameter maintaining the same average $0{\cdot}6\mu$ but the length on potato varying from $1{\cdot}0\mu$ to $1{\cdot}5\mu$, whereas on agar and in bouillon it seldom exceeded $1{\cdot}2\mu$. On potato a tandem

arrangement was frequent and in nearly all cases a marked tendency to bipolar staining was observed, especially with methylene blue. Agar cultures shewed remarkable variations between successive transfers although made at intervals of 24 hours on medium from same stock, the average length of the bacterium undergoing decided diminution (from 1·2 μ to 1·0 μ) in this short period, and in many cases again recovering the maximum length in the succeeding transfer during the following 24 hours.

The production of a diseased condition in a plant by the action of parasitic bacteria may be due to one or both of two causes. First the "wilted" condition due to interference with water-supply and loss of turgidity of the plant cells, and secondly, disorganisation and disintegration of the tissues, partly due perhaps to this interference but more directly to the action of toxins and enzymes secreted by, and characteristic of the invading organism. "Wilting" is very generally assumed to be due to the plugging of the water vessels by masses of bacteria and the purely mechanical interference with water-supply which follows; so far as the Rangpur tobacco disease is concerned my observations do not support this view; I am inclined on the contrary to attribute the wilting effect to the action of secreted toxins upon the cell protoplasm for the following reasons:—

1. Sections of the diseased stem as shewn in Plate VI made after pronounced wilting has set in, do not show a sufficiently large percentage of cells occupied by bacteria to cause any serious interference with the water-supply of the plant; a certain proportion of the cells lose their bacterial content in the process of paraffin embedding and sectioning, but comparison with thick hand-sections of fresh material shows this loss to be small.

2. A healthy tobacco plant may be cut half through the stem and a thin layer of Plasticine introduced into the cut, so as to separate the upper and lower cut ends of the vessels, without producing any symptoms of wilt even in the leaf immediately above the cut. Here the mechanical interference with the water-supply

is probably much greater than is effected by the growth of bacteria as seen in sections of the stem.

3. An alcohol precipitate from a bouillon culture of the infecting organism was dissolved in sterile water and fed into the water vascular system of healthy plants by means of a glass tube, the lower extremity of which was drawn out into a fine point, bent at right angles, and introduced into the lower part of the stem as shewn in Plate VII, Fig. 1. Control plants were similarly treated with the same solution boiled for one hour. The solution was rapidly absorbed by the plants, and wilting set in in those supplied with the unboiled solution in the course of a few days. This wilting began with the growing point, where the embryonic tissue seemed to be easily attacked, and worked backwards and downwards until the whole plant was affected. Sections of the stem near the growing point shew the characteristic disintegration of the tissues and gum formation seen in those made from plants inoculated with the bacterium (Plate VIII, Fig. 2). The control plants showed no symptoms of wilting but on the other hand seemed to appreciate the artificial supply of water.

So far as wilting is connected with failure of water-supply it seems reasonable to conclude that this failure may be due largely to the interference with osmotic pressure consequent on protoplasmic intoxication. In more advanced stages of the disease, the water-supply is no doubt further interfered with by the formation and accumulation of gum masses in the vessels.

4. Marked necrosis of the tissues takes place in the immediate vicinity of the point of inoculation, resulting in shrinkage of the tissues and formation of dark gummy masses; these are well seen in sections of the artificially poisoned plant above mentioned as shown in Plates VI, Fig. 2, and VIII, Fig. 2. It is evident that the effect of the toxins secreted by the bacteria extends to tissues in which the latter are not present. Shrinkage due to necrosis is shown in the stem (Plate VII, Fig. 2), where the pathogenic action of the bacteria is seen to be much more than

mere mechanical interference with the water-supply of the plant.

Solution of the middle lamella and consequent disintegration of the tissues by separation of the cells was a noticeable feature in sections of the stem and leaves of diseased plants; this is shewn in Plate IV, Fig. 3, and Plate IX. The peculiar reaction of the tomato plant to the influence of B. solanacearum in the formation of intumescences on the stem was noticed. Plate X shews a transverse stem section through an intumescence from which it will be seen that the latter are due to the incipient formation of aerial roots. No bacteria were to be seen in these sections. Plate XI illustrates the manner in which the course of infection in the tobacco plant takes place, the bacteria being at first confined to the particular set of water vessels into which the original inoculation took place, so that leaves homotaxially corresponding and occurring in the vertical line of inoculation are first attacked to the exclusion of intermediate ones on either side of this line. Plate XII shews the course of infection in an inoculated plant.

It seems probable that the alkalinity associated with the growth of B. solanacearum enhances its parasitic power by enabling it to overcome the unfavourable acidity of the plant juices, both with regard to the growth and multiplication of the invading organism and the solvent action upon the middle lamella of the enzyme produced by the latter.

FIELD TREATMENT.

It has been shewn by experiment in the United States (Briggs, U. S. Department of Agriculture, Bureau of Plant Industry, Circular No. 7, 4th May, 1908), that the use of mineral fertilizers which tend to produce an alkaline reaction in the soil, such as carbonate of potash, lime, and ashes, has a decidedly prejudicial effect upon the root growth of tobacco plants and renders them liable to attack by fungi; such a condition of the soil would almost certainly conduce to the multiplication of B. solanacearum, and

producing, as it has been shewn to do, a weakened root growth, would tend to facilitate the successful invasion of the plant by this parasite; although in the majority of instances the use of artificials for tobacco manuring in India is impracticable for economic reasons, it is possible that in some districts the great value of the crop might in the future lead to their use.

It has been found that this organism does not survive a temperature of 50° C. if exposed to it for as much as 30—40 minutes; thus hot weather ploughing will help to destroy a large percentage of those bacteria remaining in the surface soil and in diseased and dried up tissues, and the comparative freedom from spreading of this disease in the affected district which is, up to the present, a feature of the attack, may no doubt be largely due to this factor and should be taken into account in field practice as the most economical means of dealing with it. At the same time all diseased plants should be burnt as soon as the symptoms are noticed and not simply pulled up and thrown away. It is important that the cultivator should be impressed with the idea that each attacked plant contains the germs of the disease and that these can convey it to the soil and so to other plants of the same or following seasons. It is also important to insist upon the eradication of the whole of the plant and not merely the above ground portion.

It was found that in the case of well grown healthy plants inoculations through the root generally failed even with the most virulent cultures, which were those on potato, whereas in the case of plants recently transplanted and still suffering from disturbance of the connexion between roots and soil water, root inoculations were usually successful. On the other hand, stem inoculations did not appear to depend upon the condition of the plants so much as upon the virulence of the culture; 48 hours old cultures on potato did not fail in a single instance to produce eventual infection and finally death, when inoculated into the stem above ground with precautions against other bacterial infection, but it was found that in many cases the introduction of such saprophytes as B. megatherium or B. prodigiosus at the time of inoculation with B.

solanacearum resulted in failure of the latter to spread in the plant, which eventually overcame the invasion by the mixed culture, no doubt owing to the mutual interference between the saprophyte and the parasite.

It was found that a mixed culture of B. solanacearum and B. prodigiosus in bouillon depended for its pathogenicity upon a preponderance of the former in the medium sufficient to produce alkalinity. Such interference may no doubt in the field help to check the spread of the disease, in much the same way that the lactic acid bacillus interferes with the growth of other bacteria in sour milk, and it is probable that the individual pathogenic organisms carried over from the previous season in the soil are of a comparatively low degree of virulence and can only successfully attack plants of correspondingly low resisting power.

Such low resistance is no doubt correlated with poor root development, therefore more attention should be paid to good cultivation so as to promote a better root system: at the same time the question of water-supply depends upon proper soil treatment, as has been shewn by Howard at Pusa, where efficient hot weather cultivation results in securing a better supply of soil water for the succeeding "Rabi" crop. The local methods of cultivation which result in the formation of a surface mulch should therefore be encouraged and extended so as to affect a deeper soil stratum with the view of producing three distinct results, all of which will be in favour of the crop.

1. Deeper cultivation so as to promote extended root range and with it greater resisting power in the plant both to bacterial invasion and to drought.

2. Greater capacity of the soil for absorbing and retaining water.

3. The destruction by sun heat of a large proportion of the pathogenic bacteria, and at the same time the lowering of the numbers of nematodes by drying out the soil.

All these effects can be produced by more effective tillage without manuring, although the latter is a necessary concomitant of tobacco growing.

Particular attention should be paid to drainage; the very poor root development of all the diseased specimens as shewn in Plate X, Fig. 2, in comparison with that of a healthy and well developed plant suggests the necessity for this.

Transplanting should be done early whilst plants are still small; in this way there is less risk of injury to the plant either by loss of roots or leaves, thus avoiding the production of points of entry for bacteria and the lowering of resistance of the plant to any bacterial invasion which may occur.

A study should be made in tobacco growing districts of trap-cropping for nematodes: this is done in America by growing such plants as cowpeas and burning the crop when about 4 weeks old, thus destroying the nematodes encysted in the roots; rotation is also practised with the same object. In view of the fact that nematodes are responsible for other plant diseases in India, such a study might with advantage be extended to other crops and districts.

In general it may be said that the occurrence of this disease is probably strictly limited by the condition of the plant, so that efficient soil management is the first requirement of any system of dealing with it.

SUMMARY.

The annually recurring disease of tobacco plants in the Rangpur district of Bengal is due to infection with a bacterium of cultural characters similar to those of B. solanacearum, Smith. The symptoms are, at first, wilting of the plant, followed later by the development of dark brown streaks in the stem and midribs of the leaves, visible on the surface and found, on cutting open the former, to extend inwards to the pith and spreading upwards from the point of infection, generally about ground level, to a distance depending upon the stage to which the disease has advanced. In most cases where the diseased condition has become noticeable a greyish bacterial slime is found on cutting across the stem through the affected part.

The infecting bacterium probably is unable to gain entrance into the plant except through the intervention either of some mechanical injury or of such organisms as nematodes, which bore into the roots or collar of the plant. The operations of transplanting and intercultivation and the practice of pulling off the lower leaves may provide the former, whilst the presence of nematodes in all the plants examined in January 1913 suggests an explanation not only of bacterial infection in the fields but also in the seed beds. Field practice should aim at conservation of soil moisture and development of the root system so as to produce better grown and consequently more resistant plants: hot weather ploughing, if effectively carried out, will not only effect these two purposes but will help to keep down the numbers both of the infecting organisms and of nematodes, which latter are probably largely responsible for the entry of the former into the plant. Diseased plants should be burnt and not thrown away.

Transplanting should be carried out whilst the seedling is still small, so as to avoid undue shock to the plant and to minimise chances of damage to the roots.

Experiments should be made to discover whether the nematodes present in the soil can be diminished in number or exterminated by trap cropping, or by the use of such artificial manures as Kainit or superphosphate.

Such manures as produce an alkaline reaction in the soil should be avoided as tending to produce a lower resisting power in the plant to infection, and to increase the numbers of infecting organisms.

I wish to acknowledge my indebtedness to Mr. Shaw, Supernumerary Mycologist in this Institute, who prepared the numerous excellent paraffin sections of which photographs are given in this paper.

PUSA,
20th April 1913.

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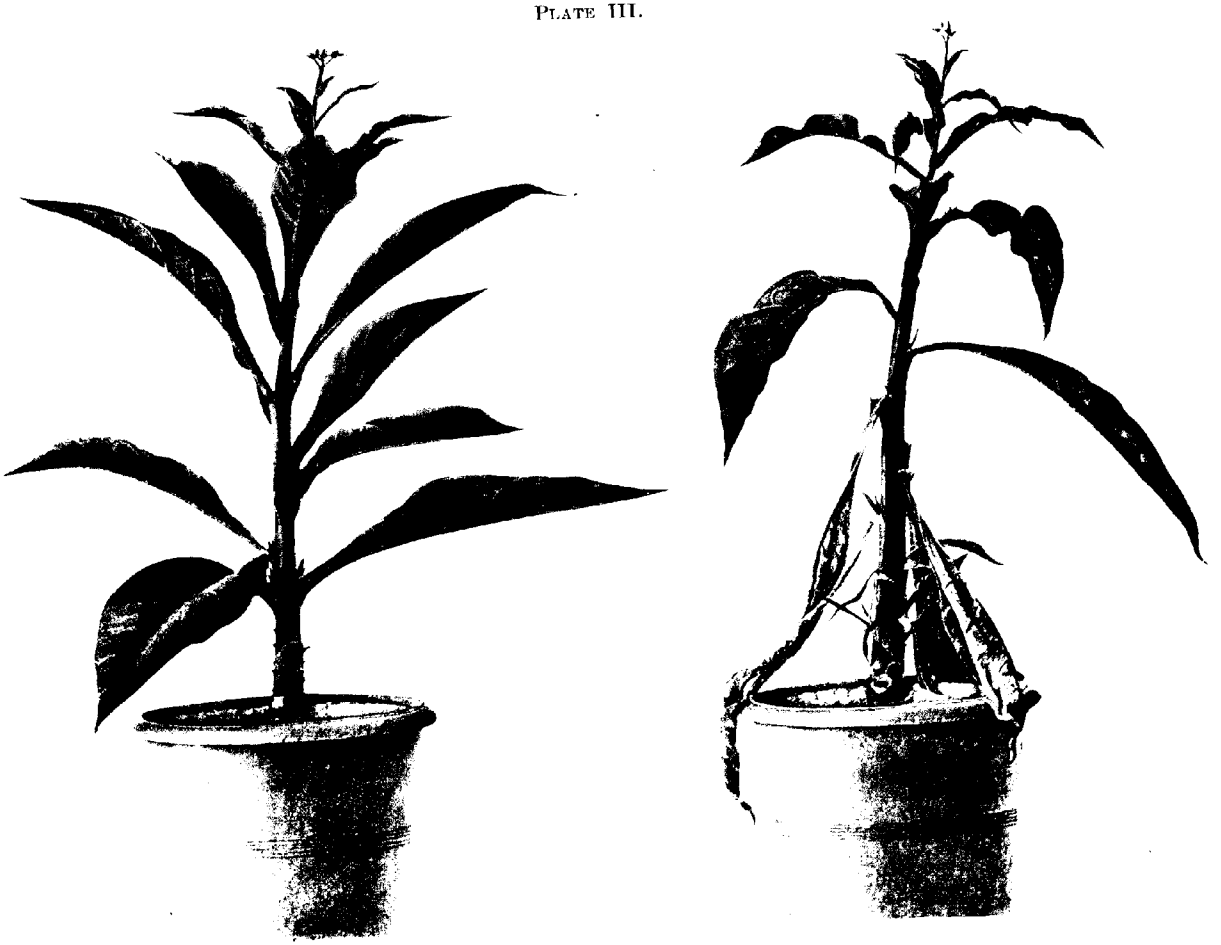
PLATE I.

Diseased tobacco plant; shewing probable point of entry of infecting organism.

PLATE II.

Tomato inoculated with B solanacearum.

PLATE III.



Inoculated tobacco plant : stages of disease.

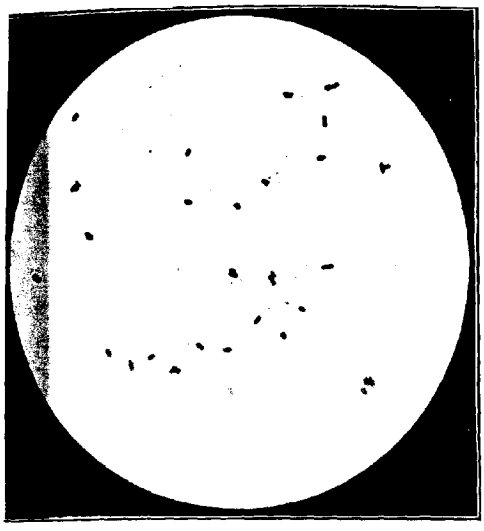


Fig. 1. B. solanacearum ; pure culture on Agar × 1,000 stained Zettnow ; no flagella. *Zeiss 2 mm. apo.*

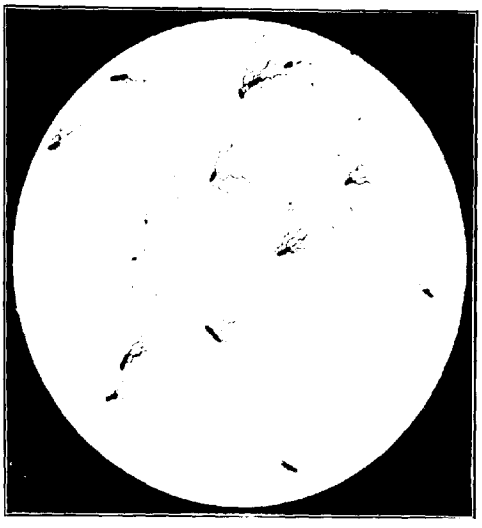


Fig. 2. Motile flagellated bacillus associated with B. solanacearum in diseased tobacco plants ; stained Zettnow. Pure culture on Agar × 1,000. *Zeiss 2 mm. apo.*

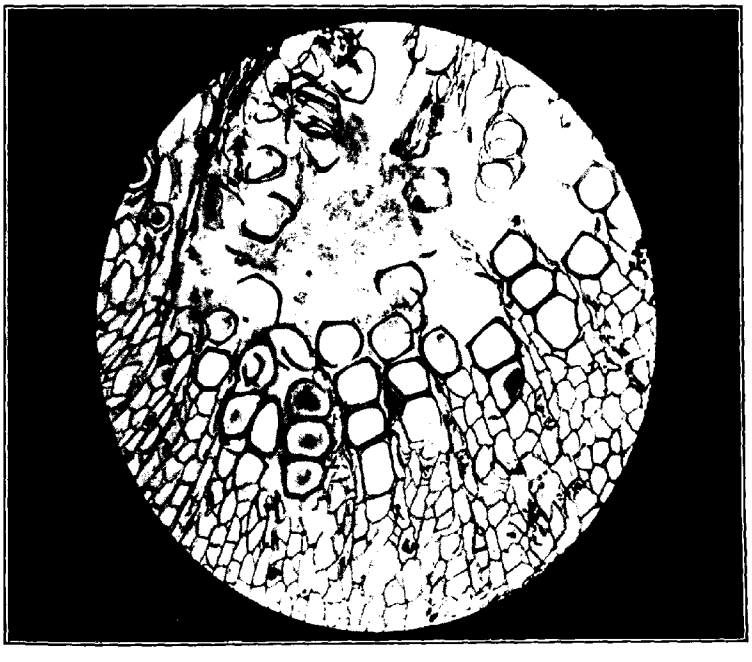


Fig. 3. Separation of cells by solution of middle lamella.

PLATE V.

Pure cultures of B. solanacearum on potato and agar.

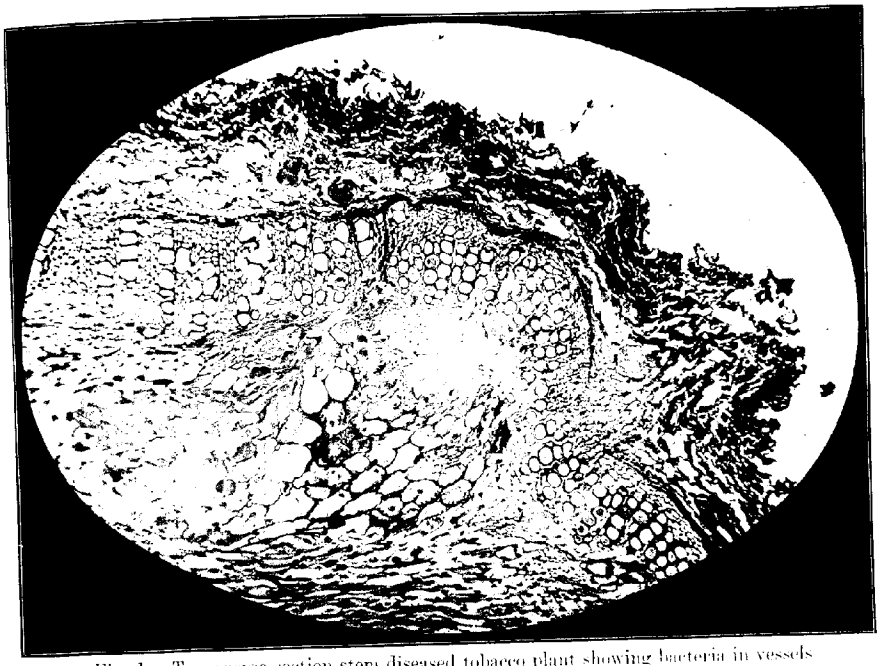


Fig. 1. Transverse section stem diseased tobacco plant showing bacteria in vessels and disintegration of tissues. *Zeiss 16 mm.*

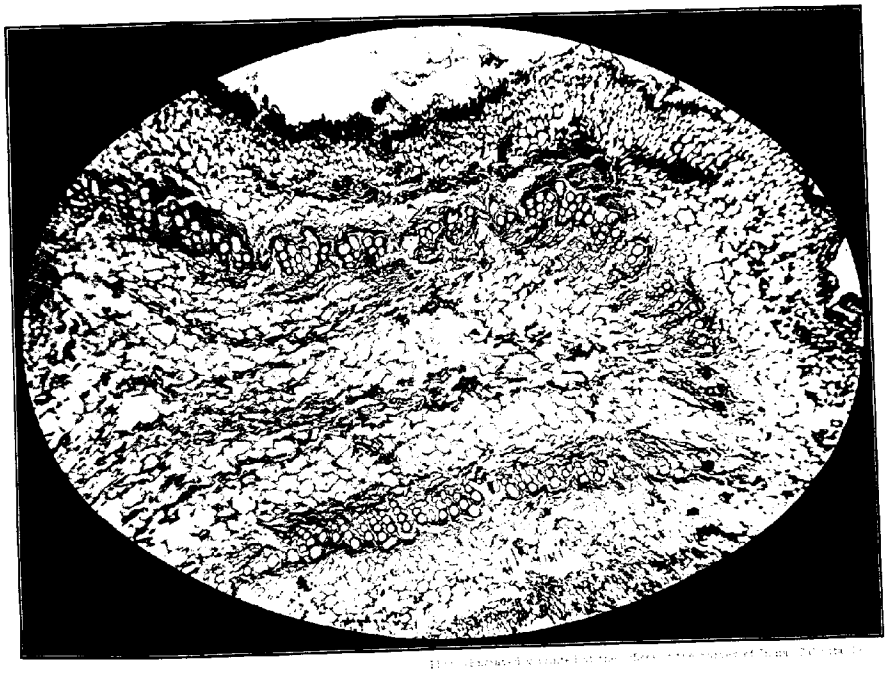


Fig. 2. Transverse section stem tobacco plant injected with toxin from culture of B. solanacearum

PLATE VII.

Wilting caused by injection of toxin.

Necrosis of stem tissues at point of inoculation A.

PLATE VIII.

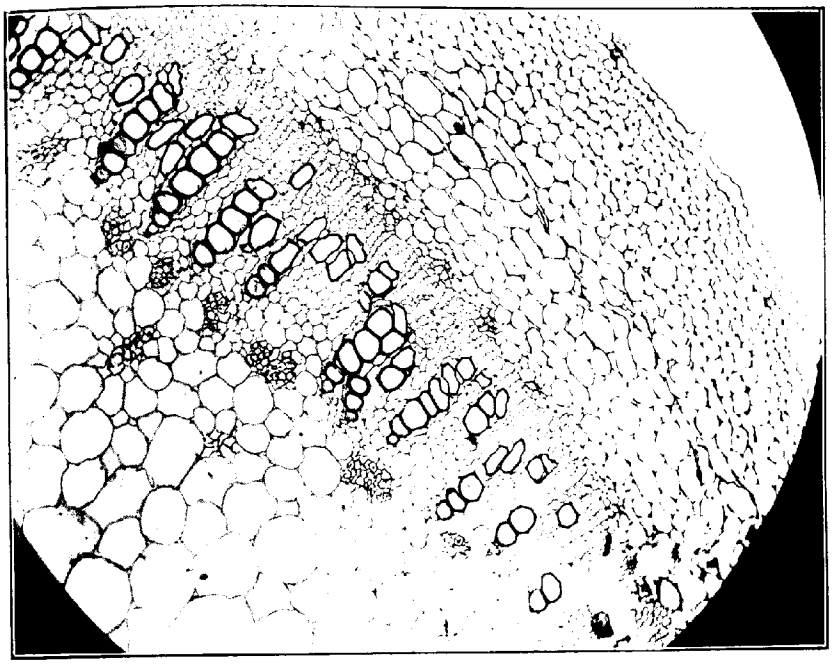


Fig. 1 TRANSVERSE SECTION OF NORMAL STEM; TOBACCO.

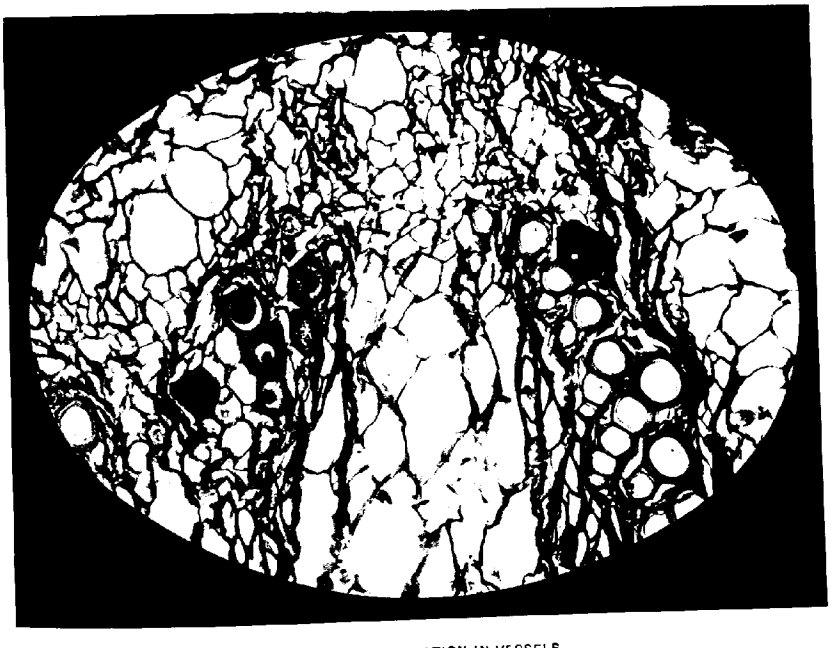


Fig. 2 GUM FORMATION IN VESSELS.

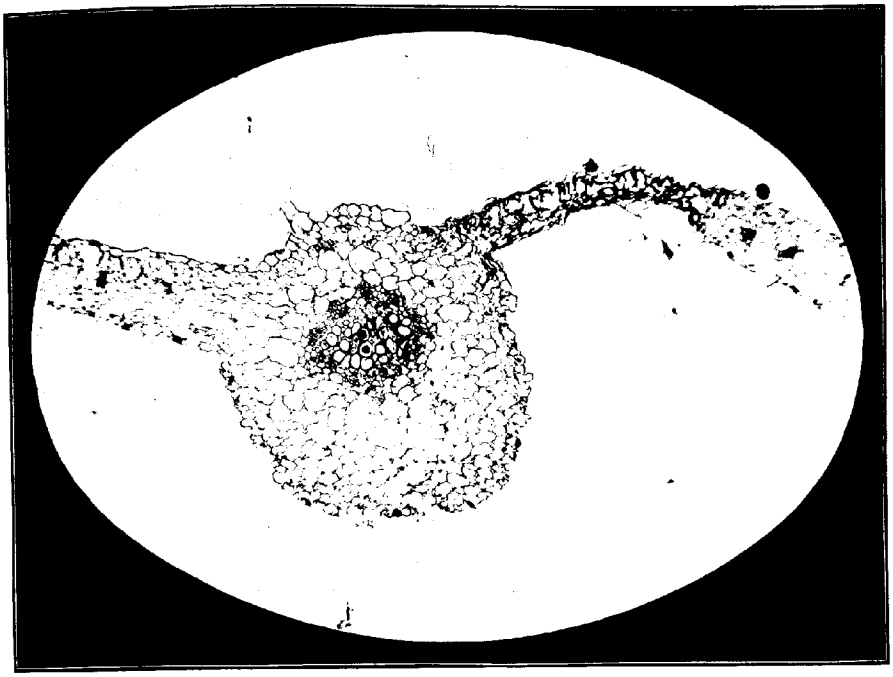
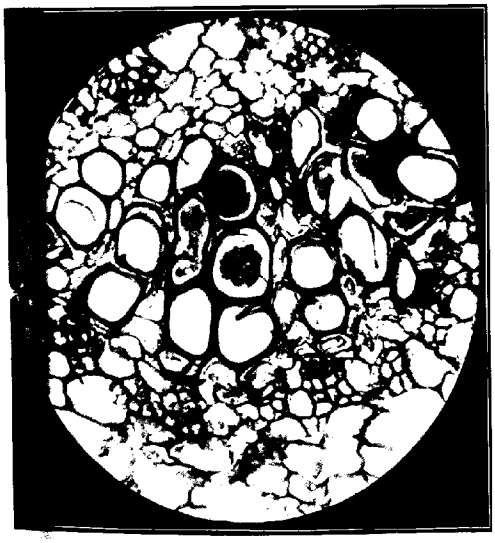


Fig. 1. Bacteria in midrib of leaf. *Zeiss 16 mm. apochromat.*



Same section. *Zeiss 4 mm. apochromat.*

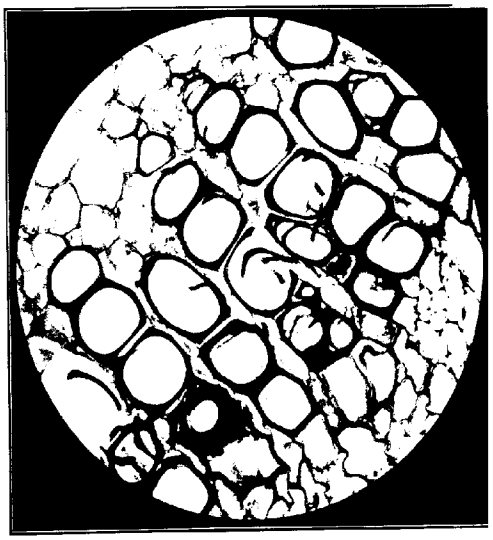


Fig. 3. Stem Transverse section showing solution of middle lamella. *Zeiss 4 mm. apochromat.*

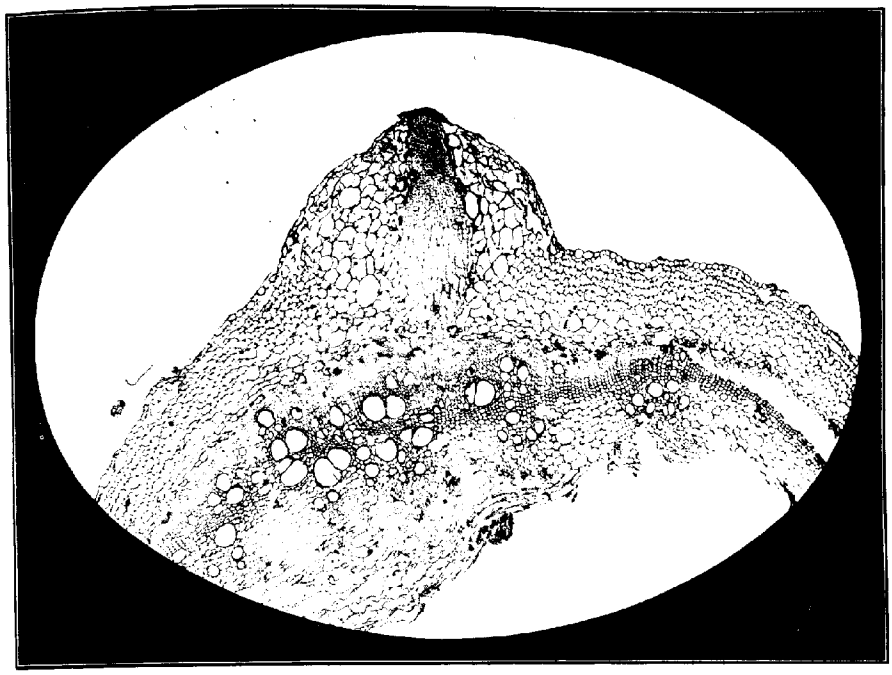


Fig. 1. Section of intumescence on tomato stem inoculated with B. solanacearum

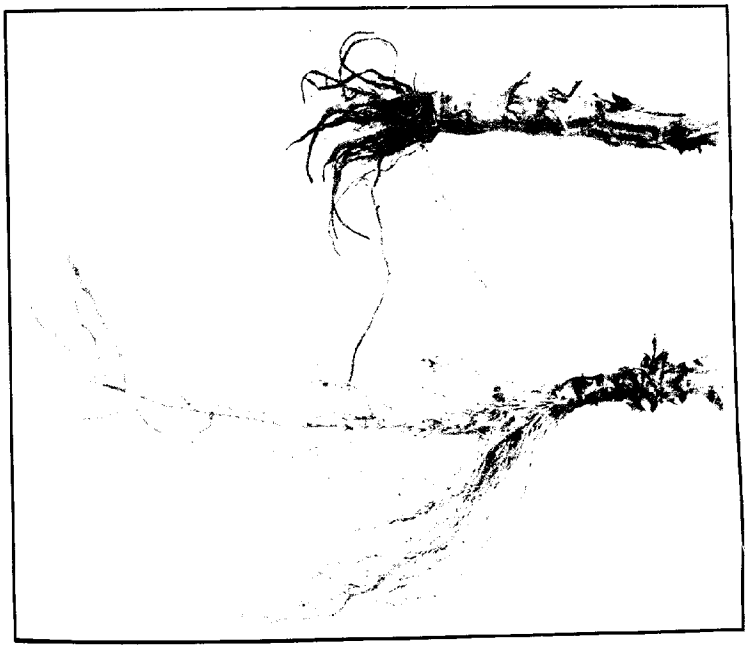


PLATE XI.

Fig. 1. Inoculated tobacco plant, showing course of infection.

Fig. 2. Growing point of tobacco plant poisoned with toxin from pure culture of B. solanacearum.

Fig. 1.
6 March 1911.

Fig. 2.
8 March 1911.

Fig. 3.
10 March 1911.

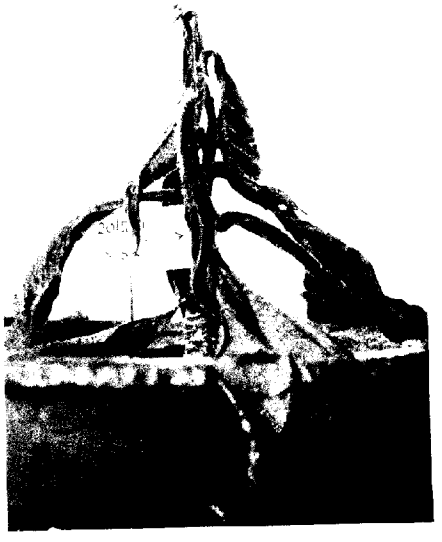


Fig. 4.
13 March 1911.

Progress of disease in plant inoculated on 20th February 1911
from pure culture of B. solanacearum on potato

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